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AMINO ACID SEQUENCE OF HUMAN CHOLINESTERASE

ANNUAL SUMMARY REPORT

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SUMMARY.

The complete amino acid sequence of human serum cholinesterase (EC 3.1.1.8) has been determined. The method used was Edman degradation of peptides purified by high performance liquid chromatography. There are 574 amino acids per subunit. Asparzines at residues 17, 106, 241, 256, 341, 455, 481, and 486 are glycosylated. The active site serine residue is located 198 amino acids from the N-terminal. The active site peptide was isolated from three different genetic types of human serum cholinesterase: from usual, atypical, and atypical-silent cholinesterases. It was found that the amino acid sequence of the active site peptide was identical in all three genotypes. Comparison of the complete sequences of cholinesterase from human serum and of acetylcholinesterase from the electric organ of Torpedo californica, shows an identity of 53%, which is a strikingly high degree of identity. Cholinesterase is of interest to the Department of Defense because cholinesterase protects against organophosphate poisons of the type used in chemical warfare. The structural results presented here will serve as the basis for cloning the gene for cholinesterase, which in turn will provide unlimited quantities of cholinesterase. The potential uses of large amounts of cholinesterase would be for cleaning up spills of organophosphate poisons and possibly for detoxification of exposed personnel.

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FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

For the protection of human subjects the investigator has adhered to policies of applicable Federal Law 45CFR46.

The investigator(s) have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements.

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INTRODUCTION

Cholinesterase detoxifies organophosphate esters by irreversibly binding the phosphate-containing portion of the ester. Cholinesterase is exquisitely sensitive to even small amounts of this poison. Its high reactivity makes cholinesterase the target for nerve gas, but also suggests its usefulness as a protective agent. For example, cholinesterase could be used to clean up spills in situations where it would be impractical to use a chemical detoxifying agent, such as sodium hydroxide, which has the drawback of being corrosive. At present cholinesterase is available in only small quantities. It is purified from human blood where only low concentrations of cholinesterase exist. To scale up the availability of cholinesterase it will be necessary to clone the gene, a project we have already initiated. Knowledge of the amino acid sequence of cholinesterase is very important to the goal of cloning the gene. The work presented here provides the amino acid sequence of human serum cholinesterase.

Prior to our results the longest sequence known for cholinesterase was an eleven-residue fragment from the active site (1). At the same time that we sequenced human cholinesterase, the laboratory of P. Taylor sequenced the acetylcholinesterase from the ray Torpedo californica (2). The results for these two cholinesterases from different sources are compared.

METHODS

Blood samples. The source of cholinesterase with the usual genotype was outdated human plasma, a gift from Dr. Harold Gallick of the Michigan Department of Public Health, Lansing. The source of atypical cholinesterase was a single female donor with a family history of succinylcholine apnea. Our atypical donor underwent plasmaphoresis over a period of two years, until 3.5 L of plasma had accumulated. Plasma was stored at -20°C . The source of atypical-silent cholinesterase was a single male donor who experienced 3 h apnea after receiving 120 mg of succinylcholine. Genotyping of 3 generations of family members revealed that he was heterozygous for atypical-silent cholinesterase. 9.4 L of his plasma were collected by plasmaphoresis over a period of 3 years.

Purification of cholinesterase. Cholinesterase was purified from outdated human plasma by ion exchange chromatography followed by affinity chromatography on procainamide-Sepharose 4B (3,4,5). The yield was 12 mg of cholinesterase from 10 liters of plasma.

Labeling cholinesterase with diisopropylfluorophosphate. The organophosphate ester, diisopropylfluorophosphate (DFP), irreversibly and specifically binds serine at the active site of cholinesterase (6). Use of radioactive DFP allows identification of the active site peptide.

To 44.7 mg of cholinesterase in 50 ml of 0.1 M phosphate buffer, pH 7, was added 2.5 ml of tritiated DFP (New England Nuclear or Amersham) containing 2.5 mCi. The amount of DFP was 526 nmoles which is one equivalent per cholinesterase active site. The mixture was incubated at 4°C for 8 days to achieve "aging". "Aging", the process in which the diisopropylphosphate derivate is catalytically converted to the monoisopropylphosphate derivative (7), was allowed to occur because we found that the aged derivate gave a single radioactive tryptic peptide. In contrast, the non-aged derivative was unstable and gave several radioactive peaks on high performance liquid chromatography (HPLC). Activity testing of the aged, DFP-labeled cholinesterase showed that 99.8% of the active sites had been labeled, since 99.8% of the activity was lost.

Reduction of disulfide bonds and alkylation with iodoacetic acid. The DFP-labeled, aged cholinesterase was reduced with 4 mM dithiothreitol in the presence of 6 M guanidine HCl, 0.1 M TrisCl, pH 8.0, for 5 hours under a constant stream of nitrogen. A 0.1 M solution of iodoacetic acid (Sigma) was freshly prepared in water, and added to the reduced protein while maintaining anaerobic conditions. The final concentration of iodoacetic acid was 9 mM. The reaction was allowed to proceed for 1 hour in the dark. The protein was desalted by dialysis against water, and then lyophilized to dryness. Cholinesterase preparations which had been labeled with DFP, and reduced and alkylated with iodoacetic acid, were used for digestion with *S. aureus* protease. For pepsin digestion a cholinesterase preparation was used that had not been labeled with DFP and had not been reduced and alkylated.

S. aureus protease digestion. 25 mg of cholinesterase was dissolved in 4 M urea, 50 mM ammonium bicarbonate, pH 7.8, and digested with 1.32 mg of *S. aureus* V8 protease (Miles Co.), at room temperature, for 48 hours.

Pepsin digestion. 25 mg of cholinesterase was dissolved in 0.4 ml of 88% formic acid and then diluted with 8 ml of water. Pepsin was from porcine stomach mucosa purchased as a highly purified enzyme from Sigma. The amount of pepsin added was 2% of the weight of cholinesterase. Digestion was at 37°C for 48 hours.

HPLC purification of peptides. Peptides were purified by HPLC. Most peptides were chromatographed three times, under 3

different solvent systems. The first solvent system was a gradient of 0.1% trifluoroacetic acid versus acetonitrile. The second solvent system was a gradient of 10 mM Na/K phosphate, pH 7.4, versus methanol. The third solvent system was a gradient of 0.1% heptafluorobutyric acid versus acetonitrile. The gradient in each HPLC run went from 0 to 60% organic solvent in 80 minutes and had a flow rate of 1 ml per minute. In some cases the second solvent system was omitted, and the peptide was chromatographed only two times before it was sequenced. Various reverse phase HPLC columns were used. For the initial digest, the most useful column was Synchropak RP-P (Synchrom Inc., Linden, IN). Its large pore size of 300 Angstroms gave excellent recoveries of even large peptides. Small peptides were further purified on a C18 Waters microbondapak column or an Altex Ultrasphere ODS 5 micron column. Other peptides were purified on Synchropak RP-P and Waters Co. phenyl column.

Manual sequencing. The manual Edman degradation procedure adapted by G. Tarr (9) for sequencing 12 to 20 peptides at once was used. In the Edman degradation method the free N-terminal of a peptide or protein is coupled with phenylisothiocyanate (Pierce Co.). The coupling serves two purposes: it attaches a colored tag to the N-terminal amino acid and it weakens the peptide bond between the terminal amino acid and adjacent amino acids so that the derivatized amino acid can be specifically cleaved off. The phenylisothiocyanate-labeled amino acid is converted to the stable derivate, the phenylthiohydantoin or PTH-amino acid. It is this form, the PTH-amino acid, that is detected at 269 nm. The modifications introduced by G. Tarr make it possible to sequence many (12 to 20) peptides at one time, and furthermore, require only small quantities (0.5 to 2 nmoles) of each peptide.

Gas phase sequencing. The manual sequencing method gave poor results after approximately 20 cycles. Therefore, peptides that were longer than approximately 20 residues were sequenced by the automated gas-phase sequencer operated by the University of Michigan Sequencing Facility.

Identification of PTH-amino acids. PTH-amino acids were identified by HPLC using the method of Black & Coon (10).

Amino acid analysis. Two commercial laboratories measured the amino acid composition of peptides. They were the AAA Labs, Mercer Island, WA, under the direction of Dr. Lowell Ericsson, and The University of Michigan Sequencing Facility, Ann Arbor, MI, under the direction of Dr. George Tarr. Salt-free peptides were hydrolyzed for 4 hours at 150°C in 6 N HCl. Amino acids were quantitated by ninhydrin at the AAA Labs, or by derivatization with phenylisothiocyanate at the University of Michigan. The latter method was developed by Tarr (11) and is now marketed by

the Waters Co. as the 'pico-tag' method. The pico-tag method was used for analyses where the amount of sample was less than 1 nmole.

cDNA library. A human adult liver cDNA library was a gift from Dr. Derek Woods of Harvard University. This library contains 250,000 independent colonies and is estimated to represent nearly all liver mRNA species with greater than 99% probability (12, 13). The cDNA in this library is joined to Pst I-digested plasmid vector pKT218 via G/C tails. The plasmid is in E. coli strain MC1061. Serum cholinesterase is made in the liver (14). Therefore a library made from mRNA of liver is expected to include cholinesterase. We estimate that the abundance of cholinesterase message in liver is low, perhaps 0.1% or less.

Oligonucleotide probes. Three different 17mer oligonucleotides were synthesized for us by P-L Biochemicals Inc., Milwaukee, WI. Each oligonucleotide was a mixture of all possible oligonucleotide chains corresponding to the selected amino acids. We selected those amino acid sequences for our probes which had the least codon ambiguity. Minimum codon ambiguity is desired because it reduces the chances of finding false positive clones. At the time the probes were synthesized we did not know where their corresponding amino acids were located in the complete sequence. Now we know that the probe regions correspond to residues 430-435, 557-562, and 347-353.

8-mix probe containing 8 oligonucleotide chains.

5' TGG CCN GAR TGG ATG GG 3' N=A,C,G,T; R=A,G
Trp Pro Glu Trp Met Gly

16-mix probe containing 16 oligonucleotide chains.

5' TGG AAR AAY CAR TTY AA 3' Y=C,T
Trp Lys Asn Gln Phe Asn

32-mix probe containing 32 oligonucleotide chains.

5' AAR GAR TTY CAR GAR GG 3'
Lys Glu Phe Gln Glu Gly

The oligonucleotides were labeled at the 5' end with gamma-³²P ATP and T4 polynucleotide kinase for use in screening the cDNA library.

Selection of positive clones and DNA sequencing. E. coli carrying the cDNA library were spread at a density of 1000 to 2000 colonies per plate, on nitrocellulose filters lying on agar

plates. The agar plates contained tetracycline. Colonies were grown to approximately 1 mm diameter. After duplication onto 2 new nitrocellulose filters and amplification on chloramphenicol containing plates, the colonies were lysed and the DNA was denatured and fixed onto the filters by the method of Grunstein & Hogness (15). The filters were hybridized with radiolabeled probes. Colonies found to be positive on duplicate filters were selected for further study.

Plasmids from positive colonies were purified by alkaline lysis (16). Plasmids were digested with Eco RI, Bam HI, or Pst I, and subjected to agarose gel electrophoresis for determination of the size of the cDNA insert. The DNA was transferred to a nitrocellulose filter by the method of Southern (17), followed by hybridization with ³²P-labeled probes. The nitrocellulose sheet was washed at increasingly higher temperatures, up to 52 °C, until only very few bands remained positive. This reduced the number of samples requiring DNA sequencing to a final total of 6 positive clones. The 6 positive clones had been selected from 600,000 colonies. DNA sequencing was by the dideoxy chain termination procedure of Sanger (18) after subcloning into M13 phage.

RESULTS AND DISCUSSION

Figure 1 gives an example of HPLC purification of peptic peptides. An earlier report (20) has HPLC traces illustrating purification of S. aureus protease peptides. Table 1 shows the amino acid sequence of S. aureus protease peptides. Table 2 shows the amino acid sequence of peptic peptides. Table 3 lists all peptides for which amino acid composition analyses were determined. These results, combined with the amino acid sequence of the tryptic peptides and the cyanogen bromide peptides reported earlier (19, 20), yield the complete amino acid sequence shown in Table 4. The individual peptides which were sequenced are indicated in Table 4 by symbols. This allows one to see the number of residues which establish an overlap.

The four amino acids, WAGV, in position 52-55 are tentative. This assignment was made on the basis of amino acid composition of a longer peptide and on homology with the Torpedo acetylcholinesterase sequence. This is the weakest part of the sequence. Two other tentative assignments are the overlap between residues 60 and 61, and the overlap between 141 and 142. Composition analyses have not yet been completed for residues 39-43, 41-60, 103-104, 133-144, and 363-368, though they are in progress. All other sequences and overlaps are supported by

numerous sequence overlaps, by amino acid composition analysis, and finally by homology with Torpedo acetylcholinesterase.

The active site serine which binds organophosphates is residue 198. The tryptic peptide containing the active site serine was sequenced from three different genetic types of human serum cholinesterase: from usual, atypical, and atypical-silent cholinesterases. All three active site sequences were found to be identical (21). Table 5 shows a comparison of the active site sequences from the three genetic types. The tryptic peptides for the atypical and the atypical-silent active site peptides are shorter than the tryptic peptide for usual cholinesterase. This result is assumed to be due to proteolysis by an unknown proteolytic enzyme which was present as a minor contaminant in the purified cholinesterases. Since the active site sequences in the three genetic types are identical, the putative amino acid mutation must be located in some other peptide that affects the activity of the enzyme. This would not ordinarily be surprising since the activity of an enzyme can be affected by residues which are near each other in 3-dimensional space but far away in a linear sequence. However, Yamato et al (1) claimed that the amino acid alteration in atypical cholinesterase was the substitution of glutamic acid (residue 197 in Table 4) for histidine. Our results leave no doubt that residue 197 is glutamic acid and is not histidine in atypical cholinesterase.

Our sequence results support our earlier conclusion (3) that the 4 subunits of cholinesterase are identical. We found only one N-terminal and only one radiolabeled active site peptide. Our peptides fit into one type of subunit and do not suggest two different subunits.

Carbohydrate chains are attached to asparagine at residues 17, 106, 241, 256, 341, 455, 481, and 486. This conclusion is based on the following observations: 1) during sequencing the residue containing carbohydrate showed up as a blank, since the carbohydrate chain prevented extraction into the sequencing solvents, 2) either threonine or serine was located two positions after the presumed carbohydrate-containing residue, 3) amino acid analysis was consistent with the presence of asparagine, 4) after acid hydrolysis at 150°C in 6 N HCl, the carbohydrate peptides had a heavy black residue caused by charring of the sugars. The finding of 8 carbohydrate chains is consistent with the report by Haupt et al (22) that 24% of the weight of human serum cholinesterase is due to carbohydrate.

Table 6 compares the sequence of human serum cholinesterase with the sequence of acetylcholinesterase from the electric organ of Torpedo californica. The amino acid sequence of the Torpedo enzyme was derived from the DNA sequence of a cDNA clone (2). The

results are surprising. The number of residues in the two enzymes is 574 and 575. This was unexpected since the subunit molecular weights are 85,000 and 70,000 daltons. From Table 6 one can conclude that the disparity in subunit weights is totally explained by a difference in the number of attached carbohydrate chains. Another unexpected finding is that 304 residues out of 574 are identical. Regions with highly conserved amino acid sequences are likely to be functionally important. The anionic site and the hydrophobic binding site for substrate are expected to be located within these regions of high homology.

In an earlier study (5) we had noted that mild trypsin digestion of purified cholinesterase caused dissociation of the tetrameric protein into monomers without affecting the apparent subunit molecular weight. We had shown that the peptide cleaved off by mild trypsin treatment contained a disulfide bond and a hydrophobic bond. The peptide was very small, and was located near one of the terminals of the subunit. We can now speculate that the disulfide bond linking two subunits is made through the cysteine at position 571, and that the hydrophobic region involved in joining subunits non-covalently includes residues 553-561.

We began searching for the cDNA clone of cholinesterase during this amino acid sequencing contract because we hoped that we could complete the sequence more quickly from the DNA sequence of the clone. This same strategy worked very well for Palmer Taylor for the Torpedo acetylcholinesterase sequence (2). It did not work for us because the abundance of mRNA for human serum cholinesterase is very low. We sequenced the DNA of 6 cDNA clones that were positive with our oligonucleotide probes, but these clones did not code for cholinesterase. The clones matched the probes in as many as 16 out of 17 nucleotides but did not match the cholinesterase sequence beyond the probe region. Our experience demonstrates that a protein present in low concentration in tissue, and presumably represented by a low concentration of mRNA, is more quickly sequenced by the classical Edman degradation procedure than by cloning.

Acknowledgment. We thank Professor Palmer Taylor of the University of California at San Diego, for showing us his amino acid sequence for Torpedo acetylcholinesterase prior to publication. This helped us complete the sequence of human serum cholinesterase more rapidly.

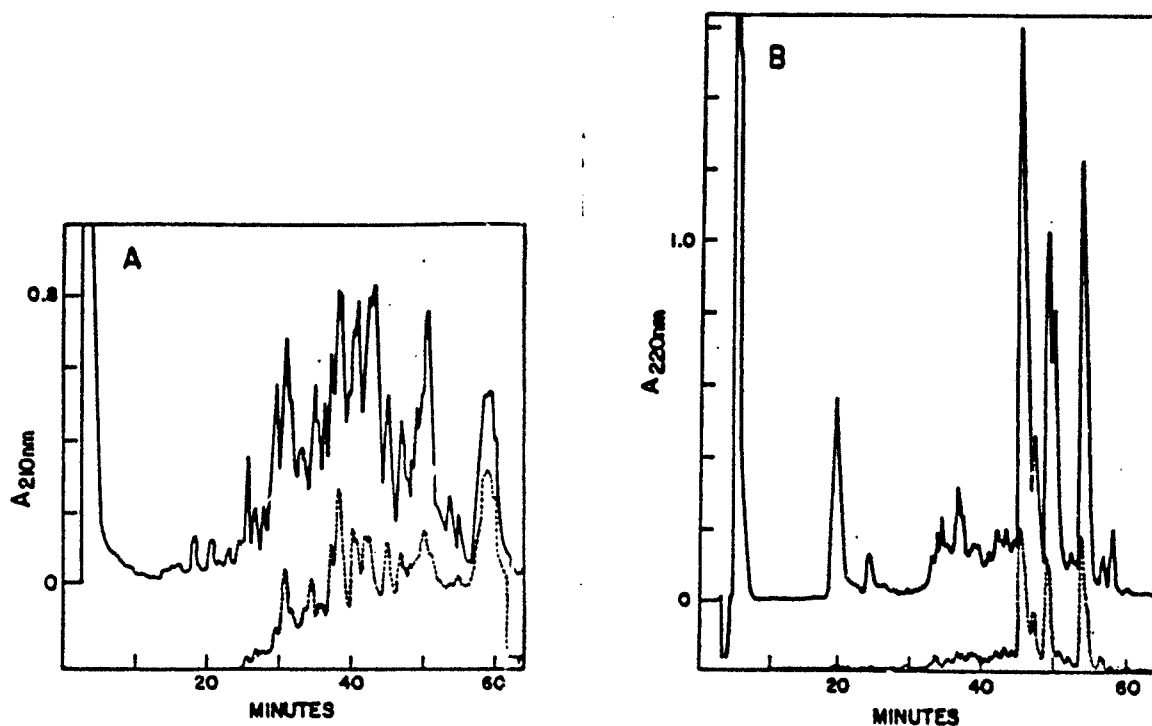


Fig. 1. HPLC separation of peptic peptides. A). 1 mg of pepsin-digested cholinesterase was injected onto a Synchropak RP-P reverse phase column which had been equilibrated with 0.1% heptafluorobutyric acid. Gradient elution was achieved by increasing the concentration of acetonitrile at a rate of 0.75% per minute. The flow rate was 1 ml per minute. The lower trace is fluorescence (stippled line); the upper trace is absorbance at 210 nm (solid line). B). The 45 minute peak from above, from a digest using 22 mg cholinesterase, was re-chromatographed on a Phenyl column (Waters Co.) which had been equilibrated in 80% solvent A (0.1% trifluoroacetic acid) and 20% acetonitrile. Gradient elution was achieved by increasing the concentration of acetonitrile at a rate of 0.3% per minute. The lower trace is fluorescence (stippled line); the upper trace is absorbance at 220 nm (solid line). The peak at 20 minutes contains 18 nmoles of peptide YEARNRTLNLAKLTGCSRENETEIIK.

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Table 1. Sequences of S. aureus protease peptides.

Location in the
complete sequence

81-90	MWNPNTDLSE
260-276	IIKCLRNKDPQEILLNE
272-276	ILLNE
277-308	AFVVPYGTPLSVNFGPTVDGDFLTDMPDILLE
326-333	GTAFLVYG
334-349	APGFSKDN*NSIITRKE
353-363	GLKIFFPGVSE
364-367	EGKE
364-374	FGKESILFHYT
368-383	SILFHYTDWVDDQRPE
384-387	NYRE
384-396	NYREALGDVVVDY
388-404	ALGDVVGDYNFICPALE
405-428	FTKKFSEWGNNAFFYYFEHRSSKL
412-422	WGNNAFFYYFE
423-432	HRSSKLPWPE
433-441	WMGVMHGYE
442-451	IEFVFGLEPLE
444-461	FVFGLEPLERRDN*YTKAEE
462-489	ILSRIVKRWANFAKYGNPN*ETQNN*STS
498-506	QKYLTLNTE
507-527	STRIMTKLRAQQCRFWTSFFP
532-540	MTGNIDEAE
539-542	AWE
543-569	WKAGFHRWNNYMMDWKNQFNDYTSKKE
570-574	SCVGL

*Carbohydrate attached to asparagine.

Peptides are designated by their location in the complete sequence of human serum cholinesterase. The numbers show the first and last position occupied.

The single letter code for amino acids is defined as follows:
A=alanine, C=cysteine, D=aspartic acid, E=glutamic acid, F=phenylalanine, G=glycine, H=histidine, I=isoleucine, K=lysine, L=leucine, M=methionine, N=asparagine, P=proline, Q=glutamine, R=arginine, S=serine, T=threonine, V=valine, W=tryptophan, Y=tyrosine.

Table 2. Sequences of peptic peptides.

Location in the
complete sequence

4-21	IIIATKNGKVRGMN*LTVF
8-28	TKNGKVRGMN*LTVFGGTVTAF
19-28	TVFGGTVTAF
24-28	TVTAF
29-51	LGIPYAQPPLRLRFKKPQSETK
81-88	MWNPNTDL
82-88	WNPNTDL
89-92	SEDC
96-110	NVWIPAPKPKN*ATVL
111-125	IWIYGGGFQTGTSSL
118-125	FQTGTSSL
126-132	HVYDGKF
133-141	LARVERVIV
142-150	VSMNYRVGA
173-189	LALQWVQKNIAAFGGNP
185-194	FGGNPKSVTL
195-206	FGESAGAAASVSL
207-220	HLLSPGSHSLFTRA
221-236	ILOSGSFNAPWAVTSL
237-262	YEARN*RTLNLAKLTGCSREN*ETEI IK
269-274	PQEIL
272-277	ILLNEA
298-306	LTDMPDIL
307-319	LELGQFKKTQILVGVNKDEGTAF
313-329	KKTQILVGVNKDEGTAF
319-329	VGVNKDEGTAF
330-354	LVYGAPGFSKD*NSI ITRKEFQEGL
364-370	FGKESIL
398-404	FICPALE
419-436	YYFEHRSSKLPWPEWMGV
434-440	MGVMHGY
440-447	YEIEFVFG
444-463	FVFG LPLERRDN*YTKAEEIL
445-463	VFG LPLERRDN*YTKAEEIL
464-475	SRSIVKRWANFA
475-493	AKYGNPN*ETQNN*STSLPVF
494-503	KSTEQKYLTL
504-521	NTESTRIMTKLRAQQCRF
526-536	FPKVLEMTGNI

*Carbohydrate attached to asparagine.

Table 3. List of Peptides Subjected to Amino Acid Analysis

Location in the
complete sequence

Tryptic peptides

1-9	EDDIIIATK
34-40	AQPPLGR
61-103	YANSCCQNIQSFPGFHGSEMWNPNNTDLSEDCLYLNWVWPAPK
106-131	N*ATVLIWIYGGGFQTGTSSLHVDGK
148-180	VGALGFLALPGNPEAPGNMGLFDQQLALQWVQK
181-190	NIAAFGGNPK
191-219	SVTLFGESAGAASVSLHLLSPGSHSLFTR
220-240	AILQSGSFNAPWAVTSLYEAR
243-248	TLNLAK
249-254	LTGCSR
266-313	NKDPQEILLNEAFVVPYGTPLSVNFGPTVDGDFLTDMPDILLELGQFK
348-355	KEFQEGLK
356-366	IFFPGVSEFGK
367-386	ESILFHYTDWVDDQRPENYR
387-407	EALGDVVG DYNFICPALETK
409-424	FSEWGNNAFFYYFEHR
428-452	LPWPEWMGVMHGYEIEFVFGLPPLER
454-458	DN*YTK
471-476	WANFAK
477-494	YGNPN*ETQNN*STSLPVFK
500-509	YLTNTESTR
521-528	FWTSFFPK
529-544	VLEMTGNIDEAEWEWK
545-549	AGFHR
559-567	NQFNDYTSK

S. aureus protease peptides

258-276	TEIIKCLRKNKDPQEILLNE
260-276	IIKCLRKNKDPQEILLNE
277-308	AFVVPYGTPLSVNFGPTVDGDFLTDMPDILLE
326-333	GTAFLVYG
334-352	APGFSDKN*NSIITRKEFQE
353-363	GLKIFFPGVSE
364-404	FGKESILFHYTDWVDDQRPENYREALGDVVG DYNFICPALE
368-382	SILFHYTDWVDDQRPE
368-404	SILFHYTDWVDDQRPENYREALGDVVG DYNFICPALE
384-404	NYREALGDVVG DYNFICPALE
405-422	FTKKFSEWGNNAFFYYFE
423-432	HRSSKLPWPE
444-461	FVFGLPLERRNN*YTKAEE
462-497	ILSRIVKRWANFAKYGNPN*ETQNN*STSLPVFKSTE
532-540	MTGNIDEAEWE
543-569	WKAGFHRWNNYMMDWKNQFNDYTSKKE
570-574	SCVGL

Table 3 continued

Peptic peptides

1-28	EDDIIIATKNGKVRGMN*LTVFGGTVTAF
111-125	IWIYGGGFQTGTSSL
126-132	HVYDGKF
173-194	LALQWVQKNIAAFGGNPKSVTL
196-203	GESAGAAS
207-216	HLLSPGSHSL
221-236	ILQSGSFNAPWAVTSL
237-264	YEARN*RTLNLAKLTGCSREN*ETEI IKCL
307-312	LELGQF
319-329	VGVNKDEGTAF
330-363	LVYGAPGFSKDN*NSIITRKEFQEGLKIFFPGVSE
398-404	FICPALE
419-436	YYFEHRSSKLPWPEWMGV
494-503	KSTEQKYLTL
504-525	NTESTRIMTKLRAQQCREWTSF
526-574	FPKVLEMTGNIDEAEWEWKAGFHRWNNYMMDW.NQFNDYTSKKESCVGL

Cyanogen bromide peptide

145-166	NYRVGALGFLALPGNPEAPGNM
---------	------------------------

*Carbohydrate attached to asparagine.

Table 4. Amino acid sequence of human serum cholinesterase.

CHO		
EDDIIIATKN GKVRGMNLTV FGGTVTAFLG IPYAQPPLGR LRFKKPQSET	50	
---T1----		--T2--
....P1.....		
.....P2.....+.....P3.....		
-----CB1-----+-----CB2-----		
CHO		
KWSDIWNATK YANSCCQNID QSFPGFHGSE MWNPNNTDLSE DCLYLNWVIP	100	
+---T3-----+-----T4-----		
.P3..		..P4...+P5.. .P6..
		////S1,////
CHO		
APKPKNATVL IWIYGGGFQT GTSSLHVDYG KFLARVERVI VVSMNYRVGA	150	
T4- ----T5-----		-T6- T7-
...P6.....+.....P7.....+P8.....+.....P9.....		
		-----CB3-----
	P10...+
CHO		
LGFLALPGNP EAPGNMGLED QQLALQWVQK NIAAFGGNPK SVTLFGESAG	200	
-----T7-----		-----T8-----T9----
	P11.....
		...P12....+P13..
-----CB3-----+-----CB4-----		
CHO		
AASVSLHLLS PGSHSLFTRA ILQSGSFNAP WAVTSLYEAR NRTLNLAKLT	250	
-----T9-----+-----T10-----+ -T11-+-		
P13..+.....P14.....+.....P15.....+.....P16.....		
CHO		
GCSRENETEI IKCLRKNKDPQ EILLNEAFVV PYGTPLSVNF GPTVDGDFLT	300	
T12+---T13---+T14+-----T15-----		
....P16.....		...P17 ..
		..P18..
		////////S2,////////+////////S3,////////+////////S4,////////
CHO		
DMPDILLELG QFKKTQILVG /NKDEGTAFL VYGAPGFSKD NNSIITRKEF	350	
-----T16-----		----
P19...+.....P20.....+.....P21.....		
///S4,///		///S5,///+////////S6,////////
-----CB5-----		

Table 4 continued

QEGLKIFFPG VSEFGKESIL FHYTDWVDDQ RPENYREALG DVVGDYNFIC 400

T17-+---T18-----+-----T19-----+-----T20-----
P22... ...P23
 ,,,,S7,,,,,+,,,S8,,,,, ,,,,S10,,,,,
 ,,,,,,S9,,,,, ,,,,S11,,,,,

PALEFTKKFS EWGNNAFFYY FEHRSSKLPW PEWMGVMHGY EIEFVFGPLPL 450

--T20-- ----T21----- ----T22-----
 ..P26....
 .P23P24..... ..P27..
 ,,+,,,S12,,,,, ,,,,S15,,,,,+,,,S16,,,
 ,,,,S13,,,,,+,,,S14,,,,, ,,,,S17,,,
 -----CB6-----

CHO CHO CHO
 ERRDNYTKAE EILSRIVKR WANFAKYGNP NETQNNSTSW PVFKSTEQKY 500

T23-+T24---+T25+ -T26-+-----T27-----+
P27.....+....P28.....+...P29.....+...P30..
 + ,,,,S18,,,,, ,,,,S19
 ,,,,S17,,,+
 -----CB6-----

LTLNTESTRI MTKLRAQQCR FWTSEFPKVL EMTGNIDEAE WEWKAGFHRW 550

-T28-----+T29-+ -T30-+-----T31-----+T32-+
 ..+...P31..... ..P32..... S22,,
 S19,,+,,,,,S20,,,,, ,,,,S21,,,,,S23,,,
 -----CB7-----

NNYMDWKNQ FNDYTSKKES CVGL 574

---T33---+T34---
 ,,,,S23,,,,,+S24,,,
 -----CB8-----

The individual peptides which were sequenced are indicated as follows: ---T--- Tryptic peptides, ...P... Peptic peptides, ,,,,S,,, S. aureus protease peptides, ---CB--- Cyanogen bromide peptides. CHO = carbohydrate chain attached to asparagine.

Table 5. Active site sequences for usual, atypical, and atypical-silent human cholinesterase genotypes.

<u>Genotype</u>	<u>Sequence</u>
	*
Usual	SVTLFGESAGAASVSLHLLSPGSHSLFTR
Atypical	SVTLFGESAGAASVSLHLLSPG
Atypical-silent	GESAGAAS

*denotes site of DFP binding.

Table 6. Comparison of the amino acid sequences of human serum cholinesterase and Torpedo acetylcholinesterase. (Torpedo data are from M.Schumacher et al, Nature, 1986.)

EDDIIIATKNGKVRGMNLTVEGGTVTAFLGIPYAQPPLGRLRFKKPQSET	50	Human
DDHSELLVNTKSGKVMGTRVPVLSSHISAF LGIPFAEPPVGNMRFR RPEPKK		Torpedo
KWAGVMQRCRYANSCCQNI DQSFPGFHGSEMWNPN TDLSEDCLYLN VWIP	100	Human
PWSGVWNA STYPNNCQQYVDEQFPGFSGSEMWNPNREMS EDCLYLN I WVP		Torpedo
APKPKNATVLIWIYGGGFOTGTSSLH VYDGKFLARVERVIVVSMNYRVGA	150	Human
SPRPKSTTVMVWIYGGGFYSGSSTLDVYNGKYLA YTEEVLVSLSYRVGA		Torpedo
LGFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESAG	200	Human
FGFLALHGSQEAPGNVGLLDQRMALQWVHDNIQFFGGDPKTVTIFGESAG		Torpedo
AASVSLHLLSPGSHSLFTRAILQSGSFNAPWAVTSLYEARNRTLNLAKLT	250	Human
GASVGMHILSPGSRDLFRRAILQSGSPNCPWASVSVAEGRRRAVELGRNL		Torpedo
GCSRENETEIIKCLR NKDPQEILLNEAFVVPYGTPLSVNFGPTVDGDFLT	300	Human
NCNLNSDEELIHCLREKKPQELIDVEWNVLPFDSIFRFSFVPVIDGEFFP		Torpedo
DMPDILLELGQFKKTQILGVNKDEGTAFLVYGAPGFSKDNN SIITRKEF	350	Human
TSLESMLNSGNFKKTQILLGVNKDEGSFLLYGAPGFSKDSESKISREDF		Torpedo
QEGLKIFFPGVSEFGKESILFH YTDWVDDQRPENYREALGDVVG DYNFIC	400	Human
MSGVKLSVPHANDLG LDAVTLQYTDWMDNNGIKNRDGLDDIVGDHNVIC		Torpedo
PALEFTKKFSEWGNNAFFYYFEHRSSKLPWPEWMGVMHG YEIEFVFG LPL	450	human
PLMHFVNKYTKFGNGTYLYFFNHRASNLVWPEWMGVIHG YEIEFVFG LPL		Torpedo
ERRDNYTKAEEILSR SIVKRWANFAKYGNPNETQNNSTSLPVFKSTEQKY	500	Human
VKELNYTAE EALSRRIMHYWATFAKTGNPNEPHSQESKWPLFTTKEQK		Torpedo
LTLNTESTRIMTKLRAQQCRFWTSFFPKVLEMTGNIDEAEWEWKAGFHRW	550	Human
IDLNTEPMKVHQRLRVQMCVFWNQFLPKLLNATETIDEAERQWKTEFHRW		Torpedo
NNYMDWKNQFNDYTSKKESCVGL	574	Human
SSYMMHWKNQFDHYSRHESCAEL		Torpedo

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